American Journal of Medical Genetics 68:282–287 (1997)

# Sterol Concentrations in Cultured Smith-Lemli-Opitz Syndrome Skin Fibroblasts: Diagnosis of a Biochemically Atypical Case of the Syndrome

**Akira Honda,[2*] G. Stephen Tint,[1,2] Gerald Salen,[1,2] Richard I. Kelley,[3] Megumi Honda,[2] Ashok K. Batta,[2] Thomas S. Chen,[1,2] and Sarah Shefer[2]**

[1] *Departments of Medicine and Pathology, Veterans Affairs Medical Center, East Orange, New Jersey*
[2] *The University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark*
[3] *The Kennedy Krieger Institute and Department of Pediatrics, Johns Hopkins Medical Institutions, Baltimore, Maryland*

**The Smith-Lemli-Opitz syndrome is a common birth defect syndrome caused by a deficiency of 7-dehydrocholesterol $\Delta^7$-reductase, an essential enzyme in the biosynthesis of cholesterol. The syndrome can usually be diagnosed easily from the plasma markers of markedly elevated 7-dehydrocholesterol and reduced cholesterol concentrations. However, atypical cases with normal plasma levels of cholesterol with only moderately elevated 7-dehydrocholesterol have been reported. To establish a sensitive method for the biochemical diagnosis of the atypical cases of the syndrome, we measured sterol concentrations of cultured skin fibroblasts. 7-Dehydrocholesterol concentrations in patients' fibroblasts grown in the presence of 10% fetal bovine serum were significantly higher than those in controls and parents ($P < 0.0005$), but they were not elevated proportionately as much as in plasma. To re-produce the accumulation of 7-dehydrocholesterol, the cells were exposed to delipidated medium to induce sterol biosynthesis. After 4 weeks, 7-dehydrocholesterol concentrations in patients' fibroblasts increased from 2.8 ± 0.3% to 34 ± 3% of total sterols (cholesterol + 7-dehydrocholesterol + 8-dehydrocholesterol). The increase was also observed in fibroblasts from an atypical patient who has a normal plasma cholesterol level and a 7-dehydrocholesterol concentration of only 0.15 mg/dl. In contrast, cells from parents and controls accumulated very little 7-dehydrocholesterol (< 1% of total sterols). These results demonstrate that cultured fibroblasts exhibit abnormally high accumulation of 7-dehydrocholesterol after cells are exposed to delipidated medium not only in typical patients, but also in an atypical case. The present method is a sensitive procedure for the biochemical diagnosis of this syndrome. Am. J. Med. Genet. 68:282–287, 1997.** 

**KEY WORDS: Smith-Lemli-Opitz syndrome; skin fibroblast; 7-dehydrocholesterol; 8-dehydrocholesterol**

## INTRODUCTION

The Smith-Lemli-Opitz (RSH) syndrome [Smith et al., 1964] is an autosomal recessive disorder characterized clinically by mental retardation, failure to thrive, and multiple congenital anomalies [Smith et al., 1964; Chasalow et al., 1985; Curry et al., 1987; Gorlin et al., 1990; Pober et al., 1990]. Recently, we reported that patients with this syndrome exhibit a combination of abnormally low levels of cholesterol in plasma and tissues and markedly increased concentrations of the cholesterol precursor, 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol), and its isomer, 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) [Irons et al., 1993; Tint et al., 1994, 1995a,b; Batta et al., 1995]. We demonstrated further that the sterol abnormality is caused by deficient activity of 7-dehydrocholesterol $\Delta^7$-reductase, an essen-

Contract grant sponsor: Department of Veterans Affairs Research Service; Contract grant sponsor: NIH; Contract grant numbers: HL 17818, HD–31932.

*Correspondence to: Akira Honda, M.D.,Ph.D., GI Laboratory, VA Medical Center, 385 Tremont Avenue, East Orange, NJ 07018-1095.

Received 18 October 1995; Accepted 31 May 1996

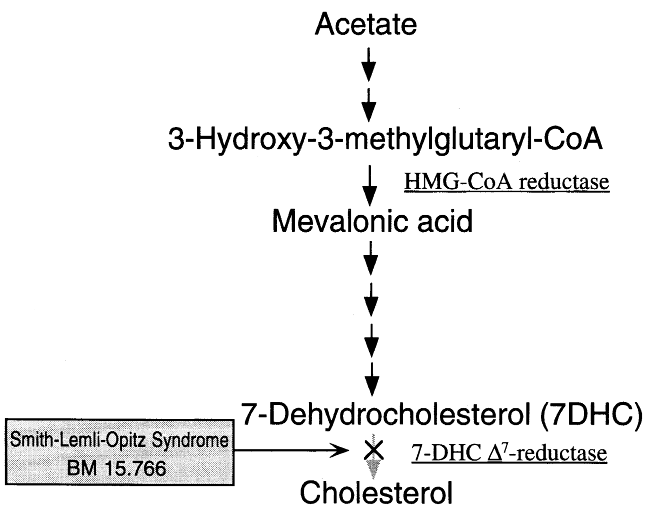


Fig. 1. Cholesterol biosynthesis from acetate. The rate-controlling enzyme is HMG-CoA reductase, whereas the defective enzyme in the Smith-Lemli-Opitz syndrome is 7-dehydrocholesterol (7-DHC) $\Delta^7$-reductase.

tial enzyme in the biosynthesis of cholesterol [Honda et al., 1995; Shefer et al., 1995] (Fig. 1).

Usually, the syndrome is diagnosed easily by demonstrating markedly increased plasma concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol, combined with low levels of cholesterol [Irons et al., 1993; Tint et al., 1994]. However, rare atypical cases exhibit normal plasma cholesterol levels with concentrations of 7-dehydrocholesterol that are considerably below that reported for most individuals with the syndrome [Kelley, 1995; Tint et al., 1995a].

Cultured skin fibroblasts from patients also manifest the same enzyme deficiency [Honda et al., 1995]. However, the concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol in fibroblasts from an affected individual that were grown in the presence of 10% fetal bovine serum (FBS) were not elevated proportionately as much as in tissue and plasma [Honda et al., 1995; Pierquin et al., 1995]. Recently, Kelley [1995] reported that the levels of 7-dehydrocholesterol in patients' fibroblasts increased markedly under cholesterol-depleted conditions. By using his method, we reproduced markedly high accumulations of 7-dehydrocholesterol in cultured skin fibroblasts from patients, including an atypical case, and established a sensitive and useful method for the diagnosis of the atypical case of the syndrome.

## MATERIALS AND METHODS

### Patients

We studied 10 patients. Five (two females and three males, ages 2–13 years) were the least clinically affected and were assigned to group I. Four other subjects (one female and three males) assigned to group II were generally younger children with clinical presentations consistent with the most profoundly affected type II phenotype [Curry et al., 1987]. Three group II patients had died by 4 weeks, 13 weeks, and 9 weeks, respectively. Patient 29, who has a normal plasma cholesterol level and a 7-dehydrocholesterol concentration of only 0.15 mg/dl, is a 25-year-old man originally described by Johnson [1975]. Of our group of 77 patients (data not shown), he is the most atypical one with the lowest plasma 7-dehydrocholesterol concentration. Although he had normal genitalia and no major organ abnormalities, he had minor clinical manifestations of the syndrome. This patient is able to speak, although his language is limited, and can walk. He attended special classes at school when he was younger. His sister is also affected. She is 31 years old and her plasma cholesterol and 7-dehydrocholesterol concentrations are 132 and 11 mg/dl, respectively.

### Fibroblast Culture

Skin biopsy specimens were obtained from patients and from their parents, and the fibroblasts were grown and maintained as monolayers in Dulbecco's Modified Eagle Medium (D-MEM) (Life Technologies, Grand Island, NY) supplemented with 10% FBS. Controls were patients from other metabolic studies without abnormalities in cholesterol metabolism. All cells were used before the 15th passage. To investigate the effect of delipidated medium on cell sterols, the following procedure was used. On day 1, 25 $cm^2$ tissue culture flasks were seeded with $3 \times 10^5$ cells/flask. On day 7, when the cells were nearly confluent, the original growth medium was removed, the attached cells were washed twice with phosphate-buffered saline (PBS), and the medium replaced with 4 ml of fresh D-MEM containing 2.5 mg/ml of delipidized protein prepared from FBS [Capriotti and Laposata, 1987]. In some experiments BM 15.766, 4-(2-[1-(4-chlorocinnamyl)piperazin-4-yl] ethyl)-benzoic acid, an inhibitor of 7-dehydrocholesterol $\Delta^7$-reductase [Aufenanger et al., 1986] (gift from Boehringer Mannheim GmbH, Mannheim, Germany), was also added (in 20 µl dimethyl sulfoxide) to the medium. Cell growth was maintained at 37°C in a humidified incubator containing 5% $CO_2$ and 95% air, and the medium was replaced with fresh delipidated medium once per week.

### Sterol Analysis

Cells for sterol quantification were harvested by trypsinization and washed twice with PBS; an aliquot was taken for determination of protein concentration [Bradford, 1976]. After the addition of 1 µg of coprostanol (5β-cholestan-3β-ol) as an internal recovery standard, lipids were extracted from the cells with chloroform-methanol 2:1 [Folch et al., 1957]. The extracts were either hydrolyzed in 1 N NaOH ethanol for 1 hour at 70°C, extracted with n-hexane, and converted into trimethylsilyl (TMS) ether derivatives [Tint and Salen, 1982] or directly converted into TMS ether derivatives without alkaline hydrolysis before analysis by GC-MS with selected-ion monitoring (SIM) using a Hewlett-Packard model 5988 mass spectrometer [Kelley, 1995; Tint et al., 1995b]. A nonpolar CP-Sil 5CB (25 m $\times$ 0.25 mm ID) capillary column (Chrompack, Raritan, NJ)

was used with a flow-rate of helium carrier gas of 1.0 ml/min. The column oven was programmed to change from 100 to 265°C at 35°C/min after a 2-minute delay from the start time. The mass spectral resolution was approximately 1000. The multiple ion detector was focused on m/z 325 for 7-dehydrocholesterol and 8-dehydrocholesterol, m/z 329 for cholesterol, and m/z 370 for coprostanol.

Plasma cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol concentrations were determined by capillary-column gas-liquid chromatography (GLC) as described previously [Batta et al., 1995; Tint et al., 1995b].

### Statistics

Data are reported here as the mean ± SEM. The statistical significance of differences between the results in the different groups was evaluated with the Student's two-tailed t test or paired two-tailed t test and significance was accepted at the level of $P < 0.05$.

## RESULTS

Table I shows the plasma sterol concentrations measured in the patients and their parents. Abnormally low cholesterol levels and high concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol were found in all patients, except for patient 29 in whom plasma cholesterol was normal and 7-dehydrocholesterol was only moderately elevated. The cholesterol levels were especially reduced in the most severely affected group II patients ($P < 0.05$, compared with group I), although 7-dehydrocholesterol and 8-dehydrocholesterol concentrations did not differ significantly between the two groups. 7-Dehydrocholesterol and 8-dehydrocholesterol were not detected in parents' plasma when our conventional gas chromatographic methods [Batta et al., 1995; Tint et al., 1994] were used.

Table II shows the cell sterols in fibroblasts grown in D-MEM containing 10% FBS. The concentrations and proportions of 7-dehydrocholesterol were significantly elevated in fibroblasts from all patients, including patient 29, compared with parents and controls, although there was no significant difference between the two groups of the patients. Compared with 7-dehydrocholesterol, little 8-dehydrocholesterol was detected in all cell lines studied.

TABLE I. Plasma Concentrations of 7-Dehydrocholesterol (7-DHC), 8-Dehydrocholesterol (8-DHC), and Cholesterol in Patients and Parents

| | Plasma concentration (mg/dl) | | |
|---|---|---|---|
| Subject (no.) | 7-HDC | 8-DHC | Cholesterol |
| Patient 29 | 0.15 | 1.0 | 154 |
| Group I patients (5) | 16 ± 4[a] | 14 ± 4[a] | 55 ± 14[a] |
| Group II patients (4) | 30 ± 11[a] | 16 ± 4[a] | 7.7 ± 2.5[a,b] |
| Parents (11) | <0.02[c] | <0.02[c] | 182 ± 13 |

[a] $P < 0.0005$, significantly different from parents.
[b] $P < 0.05$, significantly different from group I patients.
[c] Limit of detection.

Figure 2 illustrates the effects of exposure time to delipidated medium on the concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol as a percentage of total sterols in a typical patient (group II), a control, and a control grown with BM 15.766 at $5 \times 10^{-5}$ M. The plots of the accumulation of 7-dehydrocholesterol vs. incubation time from the patient and the control with BM 15.766 were similar (Fig. 2a). However, the plot from the control without inhibitor was very different. In the case of the patient and the control with BM 15.766, the proportion of 7-dehydrocholesterol increased to more than 30% by 4 weeks and to more than 40% by 12 weeks. In contrast, in the control without inhibitor, an increased proportion of 7-dehydrocholesterol was observed, but it did not exceed 1% of total sterols during 12 weeks of incubation. In all groups, the amount of 8-dehydrocholesterol increased time dependently, although the proportions were very low compared with 7-dehydrocholesterol in patients and control with added BM 15.766 (Fig. 2b).

Table III summarizes sterol composition after cells were exposed to delipidated medium for 4 weeks. The concentrations and proportions of 7-dehydrocholesterol were markedly elevated in fibroblasts from all patients, including patient 29, compared with parents and controls, although there was no significant difference between the two groups of patients. Compared with cells grown in 10% FBS, mean values of 7-dehydrocholesterol proportions in all patients' fibroblasts (patient 29, group I and II together) increased from 2.8 ± 0.3% to 34 ± 3% (N = 10) of total sterols. The ratios of esterified to total 7-dehydrocholesterol in all patients and parents were 0.37 ± 0.04 (N = 10) and 0.49 ± 0.14 (N = 4), respectively. The ratios of esterified to total cholesterol in all patients, parents, and controls were 0.32 ± 0.05 (N = 10), 0.45 ± 0.10 (N = 4), and 0.37 ± 0.17 (N = 4), respectively. These ratios were not significantly different among the groups. However, when we compared the ratios between 7-dehydrocholesterol and cholesterol in all patients by the paired Student's t test, the percentage of esterified 7-dehydrocholesterol was significantly higher than the percentage of esterified cholesterol (0.37 ± 0.04 vs. 0.32 ± 0.05, N = 10; $P < 0.005$).

## DISCUSSION

These results demonstrate that sterol composition in fibroblasts from SLOS patients reflects the deficient activity of 7-dehydrocholesterol $\Delta^7$-reductase (3β-hydroxysteroid $\Delta^7$-reductase). The concentrations of 7-dehydrocholesterol in patients' cells grown in media with 10% FBS were significantly higher than those of parents and controls. However, the elevations were not as much as in tissue [Tint et al., 1995b] and plasma because cholesterol-rich medium (with 10% FBS) markedly suppresses cholesterol biosynthesis in fibroblasts at a very early step [Brown et al., 1973].

In our experiments, after incubating cells for several weeks in delipidated medium, 7-dehydrocholesterol accumulated in patients' fibroblasts but not in cells from controls and parents. Although 7-dehydrocholesterol

TABLE II. Concentrations of 7-Dehydrocholesterol (7-DHC), 8-Dehydrocholesterol (8-DHC), and Cholesterol in Fibroblasts Grown in D-MEM Containing 10% Fetal Bovine Serum

| | Concentration in fibroblasts (μg/mg protein) | | | |
|---|---|---|---|---|
| Subject (no.) | 7-HDC | (%)[a] | 8-DHC | Cholesterol |
| Patient 29 | 1.0 | (2.2) | <0.02[b] | 45 |
| Group I patients (5) | 2.3 ± 0.7[c] | (2.9 ± 0.5[c]) | <0.02[b] | 68 ± 11 |
| Group II patients (4) | 2.6 ± 0.8[c] | (2.9 ± 0.6[c]) | <0.02[b] | 87 ± 27 |
| Parents (16) | 0.09 ± 0.02 | (0.14 ± 0.01) | <0.02[b] | 62 ± 7 |
| Controls (10) | 0.06 ± 0.02 | (0.11 ± 0.03) | <0.02[b] | 50 ± 6 |

[a] In parentheses, 7-dehydrocholesterol as a percentage of total sterols (7-dehydrocholesterol + 8-dehydrocholesterol + cholesterol).
[b] Trace amounts of 8-dehydrocholesterol.
[c] $P < 0.0005$, significantly different from parents and controls.

concentration in parents tended to be higher than in controls, it was not statistically significant. These results are similar to those reported by Kelley [1995]. Buttke and Folks [1992] replaced membrane cholesterol with other sterols and noted that the A3.01 cell line, isolated from a subject with acute T-cell leukemia, was defective in cholesterol biosynthesis at the step where lanosterol is demethylated. When they grew and subcultured the cells in serum-free media, cholesterol became virtually undetectable after several passages, whereas the proportions of lanosterol and 24,25-dihydrolanosterol rose to 25% and 75%, respectively. We also tried to subculture patients' fibroblasts in delipidated medium, but plating efficiency and growth were sometimes poor. Therefore, we exposed near confluent cells to delipidated medium and measured the changes in sterol composition within the same passage. However, long-term incubation of confluent fibroblasts without subculturing decreased cell viability, so that increased cell death and reduced concentrations of total cell protein were observed after 4 to 8 weeks in both patients' and controls' fibroblasts.

In patients, 37 ± 4% of 7-dehydrocholesterol and 32 ± 5% of cholesterol were esterified ($P < 0.005$ by paired t test). This result suggests that 7-dehydrocholesterol is at least as good a substrate for acyl-coenzyme A: cholesterol acyltransferase (ACAT) [Goodman et al., 1964] as is cholesterol.

Although plasma and tissue concentrations of 8-dehydrocholesterol (which we had previously called

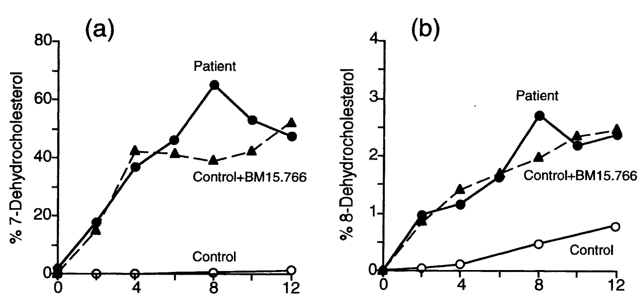


Fig. 2. Change in proportions of 7-dehydrocholesterol (**a**) and 8-dehydrocholesterol (**b**) in skin fibroblasts from a patient and control after exposure to delipidated media. Control cells were also exposed to delipidated media containing $5 \times 10^{-5}$ M of BM 15.766.

dehydrocholesterol II [Tint et al., 1994, 1995b]) in patients were often equal to or higher than that of 7-dehydrocholesterol, in all cases only trace amounts of 8-dehydrocholesterol were detected in patients' fibroblasts grown in the presence of 10% FBS. The concentrations of 8-dehydrocholesterol were increased in the patients' cells exposed to delipidated medium and in control cells exposed to delipidated medium containing BM 15.766, but they were considerably lower than those of 7-dehydrocholesterol. We have proposed that in the SLOS, plasma and tissue 8-dehydrocholesterol accumulates because of the isomerization of 7-dehydrocholesterol to 8-dehydrocholesterol [Batta et al., 1995]. This hypothesis, however, has not been proven. Our results indicate that whatever the mechanism may be, skin fibroblasts do not appear to participate in this process. In addition, Fumagalli et al. [1980] reported that treatment of pregnant rats with AY-9944, another inhibitor of 7-dehydrocholesterol $\Delta^7$-reductase, induced an accumulation of 8-dehydrocholesterol in liver of newborn rats (13% of total sterols) but not in brain (<1%), which also suggests that the production of 8-dehydrocholesterol may be confined to the liver.

Measurements of the sterol composition in fibroblasts are a useful and sensitive method for making the diagnosis of the syndrome. Although affected individuals can usually be recognized easily by the twin biochemical abnormalities of reduced plasma cholesterol and markedly elevated 7-dehydrocholesterol concentrations (Table I) [Irons et al., 1993; Tint et al., 1994], atypical patients, such as patient 29, may exhibit normal plasma cholesterol levels with concentrations of 7-dehydrocholesterol considerably below that reported for most individuals with the syndrome. Therefore, the diagnosis of the SLOS, if ascertained solely by plasma sterols, might have been missed in this subject. We recently established a sensitive biochemical method for diagnosis of the syndrome by incubating radiolabeled lathosterol with cultured skin fibroblasts, and the method easily detected the biochemical defect in patient 29 [Honda et al., 1995]. However, this is a complex method because radiolabeled lathosterol is not commercially available and both argentation and normal-phase thin-layer chromatography are needed to complete the isolation of cholesterol from lathosterol. The current study demonstrates that the biochemical defect can also be detected by measuring the 7-dehydrocho-

TABLE III. Concentrations of 7-Dehydrocholesterol (7-DHC), 8-Dehydrocholesterol (8-DHC), and Cholesterol in Fibroblasts Exposed to D-MEM With Delipidized Fetal Bovine Serum for 4 Weeks

| Subject (no.) | Concentration in fibroblasts (μg/mg protein) | | | | |
|---|---|---|---|---|---|
| | 7-HDC | (%)[a] | 8-DHC | (%)[b] | Cholesterol |
| Patient 29 | 9.7 | (35) | 0.5 | (2.0) | 17 |
| Group I patients (5) | 9.7 ± 1.7[c] | (31 ± 3[d]) | 0.4 ± 0.1[e] | (1.3 ± 0.2[e]) | 23 ± 6 |
| Group II patients (4) | 13 ± 2[d] | (38 ± 7[c]) | 0.4 ± 0.1[e] | (1.2 ± 0.1[e]) | 23 ± 6 |
| Parents (4) | 0.1 ± 0.1 | (0.4 ± 0.1) | 0.1 ± 0.1 | (0.2 ± 0.1) | 39 ± 7 |
| Controls (5) | 0.04 ± 0.03 | (0.1 ± 0.07) | 0.3 ± 0.2 | (0.7 ± 0.4) | 39 ± 6 |

[a] In parentheses, 7-dehydrocholesterol as a percentage of total sterols (7-dehydrocholesterol + 8-dehydrocholesterol + cholesterol).
[b] In parentheses, 8-dehydrocholesterol as a percentage of total sterols (7-dehydrocholesterol + 8-dehydrocholesterol + cholesterol).
[c] $P < 0.005$, significantly different from parents and controls.
[d] $P < 0.0005$, significantly different from parents and controls.
[e] $P < 0.005$, significantly different from parents.

lesterol:cholesterol ratio in fibroblasts. To diagnose the syndrome, it is not necessary to expose the cells to delipidated media, although this treatment makes the diagnosis more definitive. In addition, although we initially determined cell sterols by using a GC-MS method, after incubating the cells in delipidated medium, 7-dehydrocholesterol was detected by conventional GLC [Batta et al., 1995; Tint et al., 1995b] or ultraviolet spectrometry [Honda et al., 1997]. However, it should be noted that we could not differentiate severely affected group II patients from less affected group I patients by measuring cell sterols, whereas these groups can be distinguished based on the lathosterol conversion assay [Honda et al., 1995].

## ACKNOWLEDGMENTS

The authors thank Bibiana Pcolinsky and Nina Kovell for excellent technical assistance. This work was supported in part by grants from the Department of Veterans Affairs Research Service and by grants HL-17818 and HD-31932 from the National Institutes of Health.

## REFERENCES

Aufenanger J, Pill J, Schmidt FH, Stegmeier K (1986): The effects of BM 15.766, an inhibitor of 7-dehydrocholesterol $\Delta^7$-reductase, on cholesterol biosynthesis in primary rat hepatocytes. Biochem Pharmacol 35:911–916.

Batta AK, Tint GS, Shefer S, Abuelo D, Salen G (1995): Identification of 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) in patients with Smith-Lemli-Opitz syndrome. J Lipid Res 36:705–713.

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Brown MS, Dana SE, Goldstein JL (1973): Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins. Proc Natl Acad Sci USA 70:2162–2166.

Buttke TM, Folks TM (1992): Complete replacement of membrane cholesterol with 4,4′,14-trimethyl sterols in a human T cell line defective in lanosterol demethylation. J Biol Chem 267:8819–8826.

Capriotti AM, Laposata M (1987): Identification of variables critical to reproducible delipidation of serum. J Tissue Cult Methods 10: 219–221.

Chasalow FI, Blethen SL, Taysi K (1985): Possible abnormalities of steroid secretion in children with Smith-Lemli-Opitz syndrome and their parents. Steroids 46:827–843.

Curry CJR, Carey JC, Holland JS, Chopra D, Fineman R, Golabi M, Sherman S, Pagon RA, Allanson J, Shulman S, Barr M, McGravey V, Dabiri C, Schimke N, Ives E, Hall BD (1987): Smith-Lemli-Opitz syndrome-type II: Multiple congenital anomalies with male pseudohermaphroditism and frequent early lethality. Am J Med Genet 26:45–57.

Folch J, Lees M, Sloane Stanley GH (1957): A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509.

Fumagalli R, Bernini F, Galli G, Anastasia M, Fiecchi A (1980): The identification of a novel $C_{27}$ diene, cholesta-5,8-dien-3β-ol, in tissues of rats given AY-9944 (trans-1,4-bis(2-dichlorobenzylamino-ethyl)-cyclohexane) in pregnancy. Steroids 35:665–672.

Goodman DS, Deykin D, Shiratori T (1964): The formation of cholesterol esters with rat liver enzymes. J Biol Chem 239:1335–1345.

Gorlin RJ, Cohen MM Jr, Levin LS (1990): "Syndromes of the Head and Neck." New York: Oxford University Press, pp 890–895.

Honda A, Batta AK, Salen G, Tint GS, Chen TS, Shefer S (1997): Screening for abnormal cholesterol biosynthesis in the Smith-Lemi-Optiz syndrome: Rapid determination of plasma 7-dehydrocholesterol by ultraviolet spectrometry. Am J Med Genet 68: 288–293.

Honda A, Tint GS, Salen G, Batta AK, Chen TS, Shefer S (1995): Defective conversion of 7-dehydrocholesterol to cholesterol in cultured skin fibroblasts from Smith-Lemli-Opitz syndrome homozygotes. J Lipid Res 36:1595–1601.

Irons M, Elias ER, Salen G, Tint GS, Batta AK (1993): Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. Lancet 341: 1414.

Johnson VP (1975): Smith-Lemli-Opitz syndrome: Review and report of two affected siblings. Z Kinderheilk 119:221–234.

Kelley RI (1995): Diagnosis of Smith-Lemli-Opitz syndrome by gas chromatography/mass spectrometry of 7-dehydrocholesterol in plasma, amniotic fluid and cultured skin fibroblasts. Clin Chim Acta 236:45–58.

Pierquin G, Peeters P, Roels F, Vamos E, Brucher JM, Tint GS, Honda A, Van Regemorter N (1995): Severe Smith-Lemli-Opitz syndrome with prolonged survival and lipid abnormalities. Am J Med Genet 56:276–280.

Pober B, Curry CJR, Rutledge JC (1990): Smith-Lemli-Opitz syndrome. In Buyse ML (ed): "Birth Defects Encyclopedia." Dover, MA: Center for Birth Defects Information Services, in association with Blackwell Scientific, pp 1570–1572.

Shefer S, Salen G, Batta AK, Honda A, Tint GS, Irons M, Elias ER, Chen TC, Holick MF (1995): Markedly inhibited 7-dehydrocholesterol $\Delta^7$-reductase activity in liver microsomes from Smith-Lemli-Opitz homozygotes. J Clin Invest 96:1779–1785.

Smith DW, Lemli L, Opitz JM (1964): A newly recognized syndrome of multiple congenital anomalies. J Pediatr 64:210–217.

Tint GS, Salen G (1982): Biosynthesis of cholesterol, lanosterol, and $\Delta^7$-cholestenol, but not cholestanol, in cultured fibroblasts from

normal individuals and patients with cerebrotendinous xanthomatosis. J Lipid Res 23:597–603.

Tint GS, Irons M, Elias ER, Batta AK, Frieden R, Chen TS, Salen G (1994): Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. N Engl J Med 330:107–113.

Tint GS, Salen G, Batta AK, Shefer S, Irons M, Elias ER, Abuelo DN, Johnson VP, Lambert M, Lutz R, Schanen C, Morris CA, Hoganson G, Hughes-Benzie R (1995a): Correlation of severity and outcome with plasma sterol levels in variants of the Smith-Lemli-Opitz syndrome. J Pediatr 127:82–87.

Tint GS, Seller M, Hughes-Benzie R, Batta AK, Shefer S, Genest D, Irons M, Elias E, Salen G (1995b): Markedly increased tissue concentrations of 7-dehydrocholesterol combined with low levels of cholesterol are characteristic of the Smith-Lemli-Opitz syndrome. J Lipid Res 36:89–95.